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BIODEGRADATION OF WASTE COOKING OIL BY *Yarrowia lipolytica*

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Abstract

Waste cooking oil gets generated every day all around the world. It is commonly known as edible oil and possesses a great impact on human day-to-day life. It is commonly known as edible oil and possesses a great impact on human day-to-day life. Animals living in water consume this oil and find it hard to digest and eventually or later it dies. The usage of microorganisms, especially yeast species are being employed for degrading the waste cooking oil. The organism *Yarrowia lipolytica* was isolated and waste oil sample collected was subjected to degradation with the culture. The samples were tested for turbidity, lipase enzyme production, and chemical oxygen demand. The quality of the wastewater after biodegradation can yield non-hazardous waste cooking oil.

Keywords

Biodegradation, Chemical oxygen demand, Lipase enzyme, *Yarrowia lipolytica*, Waste cooking oil.

Introduction

Bacteria and fungi are the best decomposers in the soil environment, that helps in degradation process. Yeasts degrades the waste oil in a way that is environmentally beneficial **[1].** The microbes recycle the toxic compounds present in the environment and the end product of degradation is usually methane or oxygen. Organic components can be degraded by microorganisms either aerobically with the help of oxygen or anaerobically without the help of oxygen. Waste cooking oil is usually the frying oils used at higher temperatures, edible fat mixed in kitchen wastes, and oily wastewater directly discharged into sewage pipes that leads to blockage **(2).** Waste cooking oil contains saturated and unsaturated monocarboxylic acids. These oils go through very high temperatures-160 degrees to 200 degrees Celsius for a longer period that leads to physical and chemical changes- oxidation of fatty acids, which leads to unpleasant odour and flavour [**1**], and it is directly thrown onto wastewater flowing areas which leads to the immiscibility of cooking oil **[1]**. Microbial strains of *Pseudomonas fluroscens, Aspergillus niger*, *Humicola lanuginosa*, *Candida lipolytica*, *Chromobacterium viscosum **[3]***, *Candida rugosa*, and *Rhizopus delemar* have been used to degrade wastewater contaminated with cooking oils.

In Japan, before the year 1997, waste cooking oils were directly disposed of in the sewage systems. In China, 5 million tonnes of waste cooking oil are generated every year and 40% get into the kitchen illegally. And so, this government has encouraged the recyclers to collect the waste oil and manage it. The effective collection and disposal of waste cooking oils are a good source for the government's economic point of view. This acts as the best source of a candidate for the production of single-cell oil **[4]**, because of its low cost and its presence everywhere. During the 1970s, the Directive of Waste Oil 75/439/EEC said that the European Commission a fundamental way to collect as much waste oils from everywhere to stop the pollution to the underground as well as to improve their economy. The Waste Framework Directive 2008/98/EC deals with the topic of waste oils. Article 21 states that the waste oils have to be collected separately to ensure the protection of human health and the environment, and to stop the mixing of waste oils having different characteristics **[5]**. According to a recent report, India is the second largest buyer of cooking oil by importing. The usage of cooking oil cannot be reduced, but it can be used wisely or else managed properly. But the management of waste cooking oil is difficult in India,

1. There is no systemic disposal of cooking oil in India.
2. The waste cooking oil is disposed into rivers or waterbodies, that in turn pollutes the waterbodies and soil.
3. A single litre of oil can contaminate about one million litres of water.
4. Reuse of cooking oil is spread through generations without checking the serious health term effects.

Waste cooking oil can be used as another source (Bio-Diesel) **[4]** of energy that is produced from the microorganisms. Lipase enzymes are the bio-catalysts that are mainly used for lipid production. They are usually applied in the food industry **[6]**, drug and pharmaceutical companies **[6]**, detergent making process **[6]**, agrochemical industries **[7]** and cosmetic industries **[8]**. Microbial lipases are widely used in industries due to their outstanding properties like- the ability to the extreme temperatures, ability to work in extreme pH, and organic solvents, etc., important lipase-producing bacteria are *Pseudomonas fluorescens NS2W* **[9]10]** and *Bacillus subtilis **[11]***. Important lipase-producing fungi include *Aspergillus oryzae*.**[10]** *Saccharomycopsis* spp., **[12]** *Penicillium* spp.,***[13]*** and *Rhizopus delemar **[14]** Geotrichum candidum* **[15]**. Some yeasts also produce lipases like *Zygosaccharomyces spp.*, *Torulopsis* spp., and *Kluveromyces spp.*, The yeast strains of *Candia spp.,**[16]** Yarrowia spp., **[17]*** and *Hansenula spp.* can produce cell-bound lipases.

Materials and Methods

Microorganism and medium:

Yarrowia lipolytica, the yeast culture was isolated from Cheese and mayonnaise **[18][19]** and was cultivated on the selective medium- Glucose Yeast Peptone Medium **[20]**. This preparation was used to obtain the pure culture of the organism. For the growth of the organism, a liquid medium was prepared by adjusting the Nutrient Medium with the addition of 0.1 mg KI and 0.1mg of MgSO4 **[20][21]**. The medium was sterilized without the addition of KI and MgSO4 at 121ºC for 20 min. The sterilized vitamins were added to the liquid basal medium after cooling.

Sample and culture processing:

Three samples were collected for isolation of the organism. The samples were expiry-dated cheese, and mayonnaise collected from various possible centres. Raw milk sample **(22)** was collected on the day of the processing of the sample. A waste cooking oil sample was also collected from a local vendor where it was re-used a lot of times for the degradation process. Serial dilution was done for all the samples. 1gm and 1ml respectively to the samples were serially diluted. Standard plate count was performed for all the samples.
The medium plates stored were bought to room temperature. The organism was heavily inoculated into the liquid medium and was kept for its incubation period. Varying concentrations of oil were added into different conical flasks followed by the addition of the culture medium. The flasks were then cotton plugged and sealed with help of cling wrap. The sets of conical flasks were kept at room temperature and mixed gently regularly for the equal mixing of the culture and the oil sample. After the desired period, the samples were tested for their Chemical Oxygen Demand and Enzyme Assay.

Analytical Methods:

Chemical Oxygen Demand

The sample was centrifuged at 4000rpm for 5 minutes to separate supernatant from different contents. The supernatant was collected separately in a test tube. 20ml of the sample that was diluted to 20ml of distilled water was added to the reflux tube. The contents were mixed well so that the chlorides were converted into poorly ionized mercuric chlorides. 10ml of standard potassium dichromate solution was added. Silver sulphate was added to 30ml of sulphuric acid. This solution that contains silver sulphate and sulphuric acid was added slowly. If the color appears green in the test tube, repeat the step from first by taking smaller aliquots of the sample. The final concentration of the concentrated sulphuric acid must always be 18N. The tubes were connected to the condensers in the COD Digestor. The tubes were refluxed for 2 hours at 150 at 2 degrees Celsius. After 2 hours, the sample was cooled down. The condensers were washed with 60ml of distilled water. Titration was done. The titration was done against ferrous ammonium sulphate using ferroin as an indicator. At the end of the titration, the color changed from green-blue to wine red.

Enzyme Assay

The lipase enzyme **(23)** was determined by using olive oil emulsion technique. No surfactant was added. 0.5ml of olive oil was incubated in a shaking water bath. To that olive oil, 0.5ml of 0.1 M Calcium chloride, and 3ml of 0.6 M Phosphate buffer were added. The pH was checked and it was to be maintained at 7, neutral pH. 5ml of distilled water was added at 37 degrees Celsius. The sample was kept undisturbed for 10 minutes. 1ml of free lipase or immobilized lipase- 400-500mg was added. The sample was kept undisturbed for 20 minutes. After 20 minutes, the reaction was stopped by adding 20ml acetone, and ethanol solution at 1:1 v/v. The amount of free fatty acid was titrated with 0.02 M Sodium acetate to pH 10. Blank samples were treated similarly. One unit of lipase activity (U) is equivalent to 1 micro molecule of free acid liberated in 1 minute at 37 degrees Celsius.

Results and Discussion

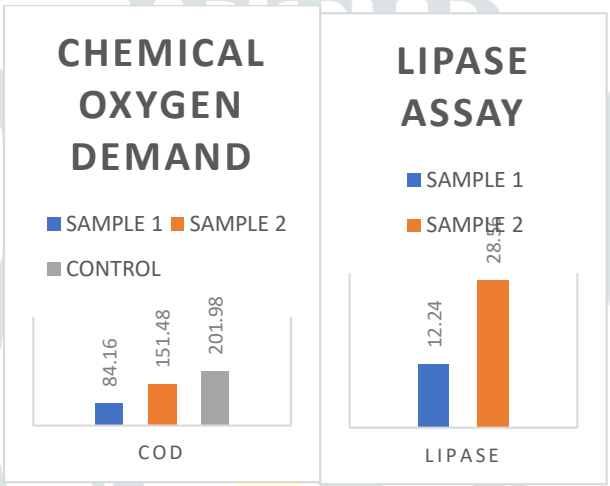
The yeast cell was spotted by performing gram staining. The cells that showed, oval shape structure, and crystal violet stain absorbed are the yeast cell. Budding cells were seen in the 100x magnification of the microscope. A germ tube test was also done to check if the organism is germ tube positive or germ tube negative. The biodegradation was done by inoculating the yeast culture into waste cooking oil. And it was kept undisturbed for a period of 12-15 days.

Table 1: Chemical Oxygen Demand

	Chemical Oxygen Demand
SAMPLE 1	84.16
SAMPLE 2	151.48
CONTROL	201.98

Table 2: Enzyme Assay

	Lipase Assay
SAMPLE 1	12.24
SAMPLE 2	28.56



The biodegradation of waste cooking oil by *Yarrowia lipolytica* gave out a product- oil that was non-pathogenic to the environment. The untreated oil in the wastewater possesses enormous health potential for humans or diseases to aquatic animals or birds. The work was done to make the oil to be biodegraded so that it can be let out in the wastewater area. The oil sample collected was subjected to biodegradation in the liquid medium with various concentrations. The samples were tested for high Chemical Oxygen Demand, Lipase Assay, and absorbance value by UV-Spectrophotometer.

The chemical oxygen demand for the control sample showed a higher result which was 201.98. This high COD value shows that the organic matter is high in amount. Whereas after the biodegradation process, there was a lot of decrease in organic matter due to the *Y. lipolytica* **(24)** and it had capability to reduce high COD value **(25**). For Sample 1, the indirect organic matter was 84.16 and for Sample 2, 151.48. If there was no decrease in the value of Chemical Oxygen Demand, it will indicate that there was no reduction in the organic matter of the sample.

The lipase assay test was done to find out the lipid content that is present extra-cellularly in the yeast *Yarrowia lipolytica*. The Lipase content for Sample 1 showed 12.24, a little production of lipids. The lipase content for Sample 2, showed 28.56, a high production of lipids.

From this study, the lowest amount of culture medium was capable to degrade a lot amount of oil sample.

Conclusion

The need for biodegradation of cooking oil is very important in upcoming years. As stated, the nature of the cooking oil changes when it has been re-used many times. This re-usage of oil poses a great question mark impact on the health of humans. Waste cooking oil when let into water areas causes serious impacts to aquatic animals. Those organisms find difficulty in breathing, feels hard to digest the food that they consume regularly and eventually the aquatic organisms die. This waste cooking oil cannot be stopped from being let out into water

areas, but the nature of the oil from being harmful to the environment can be reduced and made non-pathogenic or non-harmful to the environment by bio-degrading it.

The yeast culture was added to the oil sample and showed a good decrease in the Chemical Oxygen Demand. A little amount of culture degraded the oil sample concentration greater than the culture concentration. This shows that even little culture can degrade oil samples more than culture. The activity of culture is greater with more sample presence. This degradation activity can be carried out in bioreactors that can also have the capacity to degrade lots of oil together. This degradation makes the waste oil sample when let it to water areas to not to cause any harmful effects to the environment.

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